BECKMAN COULTER

ACCESS
Immunoassay Systems

Instructions For Use

Access TPO Antibody
Thyroperoxidase Antibody

REF A12985

FOR PROFESSIONAL USE ONLY

For *in vitro* diagnostic use

Rx Only

For use on DxI Access Immunoassay Analyzers

PRINCIPLE

INTENDED USE

The Access TPO Antibody assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of thyroperoxidase antibody (TPOAb) levels in human serum and plasma using the Access Immunoassay Systems.

The detection of TPOAb is an aid in the diagnosis of thyroid autoimmune disorders.

SUMMARY AND EXPLANATION

Detecting TPO antibodies aids in diagnosing thyroid autoimmune disorders and helps the physician distinguish between thyroid autoimmune disorders and non-autoimmune goiter or hypothyroidism.[1,2,3,4]

Disorders of the thyroid gland are frequently caused by autoimmune mechanisms with the production of autoantibodies. Thyroperoxidase (TPO) is a membrane-associated hemoglycoprotein expressed only in thyrocytes.[5] This enzyme catalyzes the oxidation of iodide on tyrosine residues in thyroglobulin for the synthesis of T3 and T4 and is one of the most important thyroid gland antigens.[6]

The determination of TPOAb levels is the most sensitive test for detecting autoimmune thyroid disease.[7] The highest TPOAb levels are observed in patients suffering from Hashimoto's thyroiditis. In this disease, the prevalence of TPOAb is about 90% of cases confirming the autoimmune origin of the disease.[2] These autoantibodies also frequently occur (60-80%) in the course of Graves' disease.

There is a good association between the presence of autoantibodies against TPO and histological thyroiditis. However, in view of the extensive regenerative capacity of the thyroid under the influence of TSH, chronic thyroid disease may be present for years before the clinical manifestation of hypothyroidism becomes evident, if ever.[8,9]

METHODOLOGY

Assay type: two-step, sandwich

The Access TPO Antibody assay is a sequential two-step immunoenzymatic (“sandwich”) assay. A sample is added to a reaction vessel with paramagnetic particles coated with thyroperoxidase protein. The serum or plasma TPOAb binds to thyroperoxidase.

After incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured

with a luminometer. The light production is directly proportional to the concentration of analyte in the sample. Analyte concentration is automatically determined from a stored calibration.

SPECIMEN

SPECIMEN COLLECTION AND PREPARATION

1. Serum and plasma (EDTA, lithium heparin) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples:[10]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Physically separate serum or plasma from contact with cells as soon as possible.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter have been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.
5. Thaw samples no more than three times. Avoid assaying lipemic or hemolyzed samples.

REAGENTS

CONTENTS

Access TPO Antibody Reagent Pack

Ref. No. A12985: 100 determinations, 2 packs, 50 tests/pack

The same reagent formulation is used on all Access Immunoassay Systems.

- All antisera are polyclonal unless otherwise indicated.

Well	Contents	Ingredients
R1a:	3.25 mL	Dynabeads* paramagnetic particles coated with streptavidin and coupled to biotinylated human recombinant TPO, suspended in a ACES buffer with protein (bovine), < 0.1% sodium azide and 0.1% ProClin** 300.
R1b:	9.6 mL	Recombinant Protein A-alkaline phosphatase (bovine) conjugate in MES buffer with protein (bovine) < 0.1% sodium azide and 0.1% ProClin 300.
R1c:	3.1 mL	TRIS buffer with protein (bovine), < 0.1% sodium azide and 0.1% ProClin 300.

*Dynabeads is a registered trademark of Dynal A.S., Oslo, Norway.

**ProClin is a trademark of LANXESS Corp.

WARNING AND PRECAUTIONS

- For *in vitro* diagnostic use.

- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

  For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

> ⚠ **CAUTION**
>
> **Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).**
> **To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.**

GHS HAZARD CLASSIFICATION

PMP (Compartment R1a)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Conjugate (Compartment R1b)	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		2-Methyl-4-isothiazolin-3-one < 0.05%

Blocking Agent for Part A (Compartment R1c)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Blocking Agent for Part B (Compartment R1c)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

If product manufacture date is September 29, 2024 or earlier use part A of SDS.
If product manufacture date is on or after September 30, 2024, use part B of SDS.

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access TPO Antibody Calibrators
   Provided at zero and approximately 5, 20, 75, 300 and 1,000 IU/mL.
   Ref. No. A18227
2. Quality Control (QC) materials: commercial control material.
3. Lumi-Phos PRO

Ref. No. B96000

4. UniCel DxI Wash Buffer II
   Ref. No. A16793
5. Optional materials for dilution:
   - Access Sample Diluent A
     - Vial Ref. No. 81908
     - Diluent Pack Ref. No. A79783

REAGENT PREPARATION

Provided ready to use.

REAGENT STORAGE AND STABILITY

Stability	
Unopened at 2 to 10°C	Up to stated expiration date
After opening at 2 to 10°C	56 days

- Store upright.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Signs of possible deterioration are a broken elastomeric layer on the pack or quality control values out of range.
- If the reagent pack is damaged (e.g., broken elastomer), discard the pack.

CALIBRATION

CALIBRATION INFORMATION

Active calibration is required for all tests. Calibration is required every 56 days. See calibrator Instructions for Use (IFU) for additional calibration information. Refer to the appropriate system manuals and/or Help system for information on calibration method, configuring calibrators, calibrator test request entry, and reviewing calibration data.

QUALITY CONTROL

Quality control materials are essential for monitoring the system's performance. Quality controls with varying concentration ranges should be run individually at least once every 24 hours when the assay is being performed.[11] Quality control ranges should be determined by each laboratory's individual requirements. Follow applicable regulations and guidelines for quality control.

TESTING PROCEDURE(S)

PROCEDURE

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

3. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
4. Use 10 µL of sample for each determination in addition to the sample container and system dead volumes. Use 50 µL of sample in addition to the sample container and system dead volumes for each determination run with the automated dilution feature. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
5. The system default unit of measure for sample results is IU/mL.

LIMITATIONS

1. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce human anti-animal antibodies, e.g., HAMA, that interfere with immunoassays. Additionally, other antibodies such as human anti-goat antibodies may be present in patient samples.[12,13] Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
2. Other potential interferences in the sample could be present and may cause erroneous results in immunoassays. Some examples that have been documented in literature include rheumatoid factor, fibrin, endogenous alkaline phosphatase, exogenous alkaline phosphatase (e.g., asfotase alfa, Strensiq), and proteins capable of binding to alkaline phosphatase. Carefully evaluate results if the sample is suspected of having these types of interferences.[14,15]
3. The results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.
4. The Access TPO Antibody assay does not demonstrate any “hook” effect up to 10,000 IU/mL.
5. The test result in and of itself is not diagnostic for thyroid disease and should be considered in conjunction with iodine uptake and other standard thyroid tests and the clinical presentation of the patient.
6. Moderately increased levels of TPO antibody may be found in patients with non-thyroid autoimmune disease such as pernicious anemia, type I diabetes mellitus, or other disorders which activate the immune system.[7,16,17]

RESULTS INTERPRETATION

Test results are determined automatically by the system software. Test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

REPORTING RESULTS

MEASURING INTERVAL

Approximately 0.25 – 1,000 IU/mL

Automated dilution: Up to approximately 10,000 IU/mL

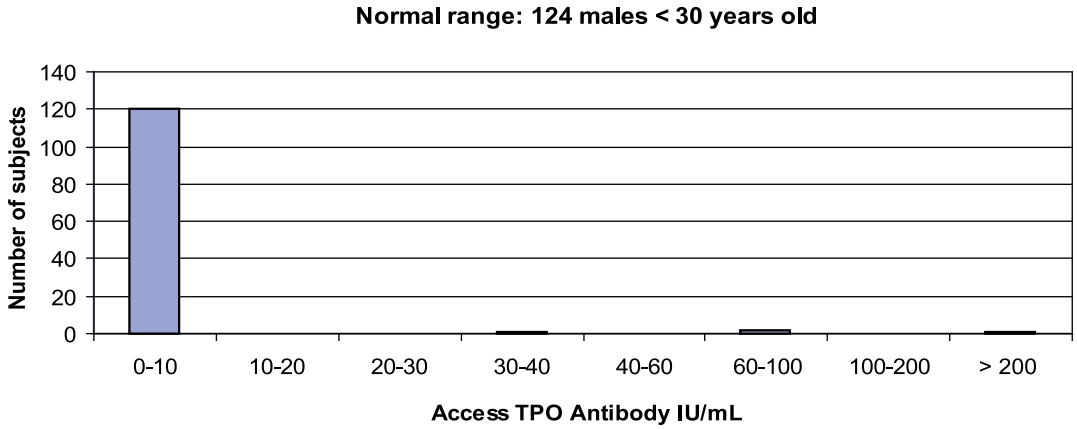
Samples can be accurately measured within the measuring interval defined as the lower Limit of Detection (LoD) and the highest calibrator value.

1. If a sample contains less than the lower limit for the assay, report the result as less than that value (i.e., < 0.25 IU/mL).
2. If a sample contains more than the stated value of the highest calibrator, report the result as greater than that value (e.g., > 1,000 IU/mL). Alternatively, the sample may be diluted to obtain a result.
   - For automated dilutions, the system dilutes one volume of sample with 9 volumes of Sample Diluent A. Refer to the appropriate system manuals and/or Help system for instructions.

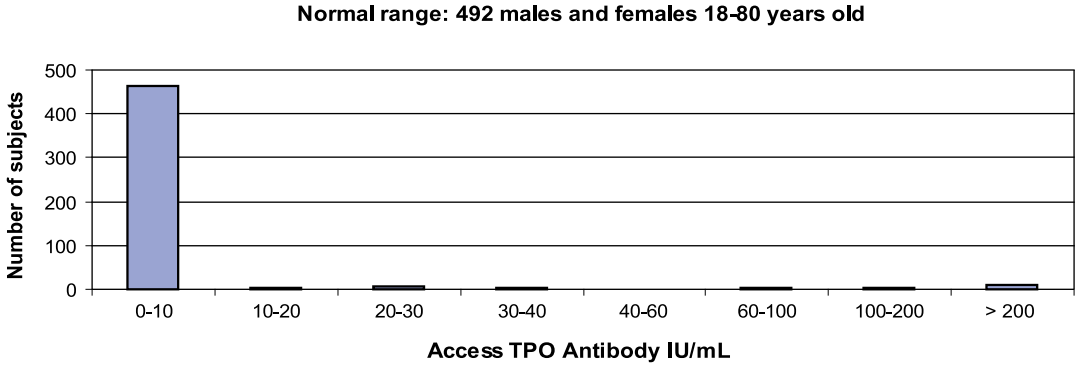
- For manual dilutions, dilute one volume of sample with 9 or 99 volumes of Sample Diluent A. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.
- Due to varying antigen specificity, affinity and avidity of thyroperoxidase antibodies in their epitope reactions some samples may not dilute linearly.[18]

EXPECTED VALUES

1. Each laboratory should validate or establish its own reference intervals to assure proper representation of specific populations.
2. Sera samples were obtained in the United States from 166 males < 30 years of age following the criteria outlined by the National Academy of Clinical Biochemists (NACB) for establishing a normal reference range for thyroid antibody tests.[9] The screening criteria included serum TSH levels between 0.5 and 2.0 mIU/L, no goiter, no personal or family history of thyroid disease, and absence of non-thyroid autoimmune disease. After completing the screen, 124 samples were tested generating a 95% non-parametric upper reference limit below 9 IU/mL.



3. Additionally, 679 normal samples were collected in the United States from both males and females ranging in age from 18-80 years old. The screening criteria included serum TSH levels between 0.5 and 2.0 mIU/L, no goiter, no personal or family history of thyroid disease, and absence of non-thyroid autoimmune disease. After completing the screen, 492 samples were tested. 93% of these samples fell below 9 IU/mL.



4. As observed through the two studies outlined above, an apparently healthy subject may have increased levels of TPO antibodies with no prior history of thyroid disease.[9]

PERFORMANCE CHARACTERISTICS

ASSAY CRITERIA AND REPRESENTATIVE DATA

Representative data is provided for illustration only. Performance obtained in individual laboratories may vary.

METHODS COMPARISON

A study based on CLSI EP09c, 3rd Edition[19] using Passing-Bablok regression and Pearson's correlation compared the Access 2 Immunoassay System and the DxI 9000 Access Immunoassay Analyzer.

N	Concentration Range* (IU/mL)	Slope	Slope 95% CI	Intercept	Intercept 95% CI	Correlation Coefficient R
219	0.35 - 980.9	1.06	1.04 - 1.08	-0.26	-0.32 - (-0.22)	0.978

*Range is Access 2 values

LINEARITY

A study based on CLSI EP06-Ed2[20] performed on the DxI 9000 Access Immunoassay Analyzer determined the assay demonstrated linearity across the measuring interval.

IMPRECISION

The assay was designed to have within-laboratory imprecision as listed below:

- < 0.07 IU/mL SD at concentrations < 0.6 IU/mL
- < 12.0% CV at concentrations ≥ 0.6 IU/mL and < 450.0 IU/mL
- < 15.0% CV at concentrations ≥ 450.0 IU/mL

A study based on CLSI EP05-A3[21] performed on the DxI 9000 Access Immunoassay Analyzer tested multiple samples in duplicate in 2 runs per day for a minimum of 20 days.

Concentration (IU/mL)			Repeatability (Within-Run)		Between-Run		Between-Day		Within-Laboratory (Total)	
Sample	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	80	0.35	0.02	6.9	0.00	0.0	0.00	0.0	0.02	6.9
Sample 2	80	5.5	0.36	6.7	0.00	0.0	0.00	0.0	0.36	6.7
Sample 3	80	20	1.12	5.6	0.00	0.0	0.52	2.6	1.23	6.2
Sample 4	80	318	18.59	5.8	0.13	0.0	10.50	3.3	21.35	6.7
Sample 5	80	747	77.4	10.4	52.21	7.0	24.18	3.2	96.48	12.9

ANALYTICAL SPECIFICITY / INTERFERENCES

Samples containing up to 40 mg/dL bilirubin, lipemic samples containing the equivalent of 3,000 mg/dL triolein (triglycerides), and hemolyzed samples containing up to 500 mg/dL hemoglobin do not affect the concentration of thyroperoxidase antibodies assayed. In addition, samples with 6 g/dL human serum albumin added to the endogenous albumin in the samples do not affect the concentration of thyroperoxidase antibodies assayed.

The following table describes the interference of the assay with commonly used medications.

Substance	Concentration	Interference (%)
Acetaminophen	0.2 mg/mL	-1.6
Acetylsalicylic acid	50 mg/dL	1.8
Ibuprofen	40 mg/dL	3.3
Heparin	8,000 IU/dL	-3.0
Multivitamins	1:20 dilution	-4.6

CLINICAL SENSITIVITY

The Access TPO Antibody assay was further evaluated using sera obtained from 54 patients diagnosed with Hashimoto's Thyroiditis and 40 patients diagnosed with Graves' Disease. The diagnosis of Hashimoto's Thyroiditis and Graves' Disease was based upon criteria established by the individual laboratory. The presence of TPO antibodies was not a criteria for disease diagnosis.

	Number of Patients	Access TPO Antibody % Positive
Hashimoto's Thyroiditis	54	100%
Graves' Disease	40	77.5%

DETECTION CAPABILITY

Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) studies were conducted on the DxI 9000 Access Immunoassay Analyzer following CLSI guideline EP17-A2.[22] The LoB study included 2 reagent lots and 2 instruments over a minimum of 3 days. The LoD and LoQ studies included 3 reagent lots, 3 instruments, and 2 calibrator lots over a minimum of 5 days.

	IU/mL
Limit of Blank (LoB)	0.19
Limit of Detection (LoD)	0.23
Limit of Quantitation (LoQ) ≤ 20% within-lab CV	0.25

ADDITIONAL INFORMATION

REVISION HISTORY

Revision A

New release of DxI Access Immunoassay Analyzer reagent IFU.

Revision B

Updated "Reagents" section.

Updated "Testing Procedure(s)" section.

SYMBOLS KEY

Glossary of Symbols is available at beckmancoulter.com/techdocs (document number C02724).

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